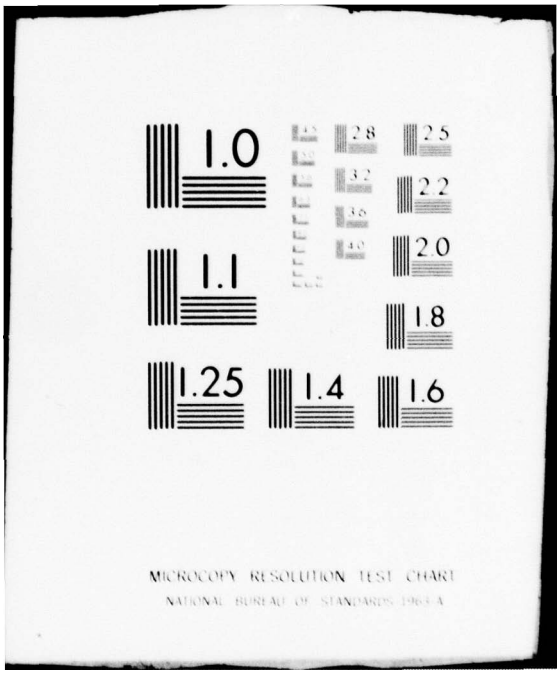
AD-A069 009 PENNSYLVANIA UNIV PHILADELPHIA DEPT OF ANIMAL BIOLOGY F/G 6/15
DEVELOPMENT OF ASSAY BASED ON EFFECTS OF PGBX ON THE ISOLATED P--ETC(U)
MAR 79 C E ARONSON N00014-78-C-0202
UNCLASSIFIED NL

| OF |
AD A069009

END
DATE FILMED
6-79
DDC


1.0
1.1
1.25
1.4
1.6
2.8
3.2
3.6
4.0
2.5
2.2
2.0
1.8
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963-A

OFFICE OF NAVAL RESEARCH
Contract N00014-78-C-0202
Task No. NR 207-125

ANNUAL REPORT NO. 1

Development of Assay Based on Effects of PGB_x on the Isolated Perfused Rat Heart

by

Carl E. Aronson, Ph.D.

Prepared and Accepted (Part I) for Publication
in
General Pharmacology

University of Pennsylvania School of Veterinary Medicine
Laboratories of Pharmacology & Toxicology
Department of Animal Biology
Philadelphia, PA 19104

1 March 1979

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: Annual Report No. 1
2. GOVT ACCESSION NO.:
3. RECIPIENT'S CATALOG NUMBER:

4. TITLE (and Subtitle): Development of Assay Based on Effects of PGB_x on the Isolated Perfused Rat Heart
5. TYPE OF REPORT & PERIOD COVERED: Annual 3/78 - 2/79
6. PERFORMING ORG. REPORT NUMBER:

7. AUTHOR(s): Carl E. Aronson, Ph.D.
8. CONTRACT OR GRANT NUMBER(s): N00014-78-C-0202

9. PERFORMING ORGANIZATION NAME AND ADDRESS:
~~Laboratories of Pharmacology & Toxicology~~
Department of Animal Biology
University of Pennsylvania School of Vet. Med.
Philadelphia, PA 19104
10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 28 p.

11. CONTROLLING OFFICE NAME AND ADDRESS:
Office of Naval Research
Biological Sciences Division - Biophysics
Arlington, VA 22217
12. REPORT DATE: 1 Mar 1979
13. NUMBER OF PAGES: 28

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office):
15. SECURITY CLASS. (of this report): Unclassified
15a. DECLASSIFICATION/DOWNGRADING SCHEDULE:

16. DISTRIBUTION STATEMENT (of this Report)
Unlimited
Annual rept. no. 1, Mar 78 - Feb 79

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES
Part I accepted for publication in *General Pharmacology*

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Adenine nucleotides	Electrical activity	Spontaneous heart rate
Biochemical activity	Glycolytic intermediates	
Coronary flow	Isolated perfused rat heart	
Diastolic tension	Isometric systolic tension	
Disophenol	PGB_x	

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
PART I
1. PGB_x at high concentrations (≥500 ng/ml) depressed isometric systolic tension and coronary flow and decreased tissue concentrations of ATP and creatine phosphate. —> over

—> PGB(x), a polymeric base-catalyzed derivative of 9,15-diketoprostaglandin E

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE
S/N 0102-LF-014-6601

Unclassified
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

411 187

20. (continued)

2. At 1000 ng/ml, PGB_x produced similar effects, but in addition, it markedly enhanced cardiac glycogen utilization, increased tissue concentrations of glucose-1-phosphate and glucose-6-phosphate and caused a significant increase in diastolic tension after being perfused for 60 minutes at this concentration.

PART II

1. In well-oxygenated perfused rat hearts, PGB_x had no effect on disophenol induced alterations in spontaneous heart rate, but did appear to prevent the increase in coronary flow caused by disophenol.

2. Isometric systolic tension decreased significantly in PGB_x-disophenol treated hearts but not in those receiving disophenol alone. Diastolic tension was increased in hearts which received PGB_x and disophenol simultaneously.

3. Disophenol caused alterations in glycogen and adenine nucleotide concentrations which were uninfluenced by PGB_x. The amount of ADP in the tissue, however, was lower in those hearts which received both PGB_x and disophenol. Phosphorylase activity and the lactate present in coronary effluent was the same in both groups.

TABLE OF CONTENTS

*The data in Part I has been accepted for publication in *General Pharmacology*

PART I

INTRODUCTION

PGB_x, a polymeric base-catalyzed derivative of 9,15-diketoprostaglandin E, was first described and shown *in vitro* by Polis *et al* (1973) to reverse degenerative changes in mitochondria. In their system, addition of PGB_x restored oxidative phosphorylation and ATP values to normal in aged mitochondria (prepared from rat liver) whereas the untreated mitochondria showed further deterioration. These findings have since been confirmed by Devlin (1978). The ability of PGB_x to restore "normal" biochemical function in damaged mitochondria appears unique, and the drug shows promise of being of value in situations such as myocardial infarction, shock and acceleration stress where hypoxia of critical tissues and mitochondrial damage may possibly occur.

In monkeys, PGB_x had a protective effect against the mortality caused by coronary ligation and subsequent ventricular fibrillation, and mitochondria from infarcted areas of heart in PGB_x-treated animals showed minimal degenerative changes compared to those from untreated controls when examined by transmission electron microscopy (Riley *et al*, 1974, Angelokos *et al*, 1977).

Since little is known about the effects of the drug on normal tissue and its protective/stimulating effects on mitochondria appear to occur only in degenerating preparations (Riley *et al*, 1974), it was decided to investigate the effect of PGB_x on the isolated perfused rat heart, an *in vitro* system utilizing an intact organ (Aronson and Serlick, 1976, 1977a,b). The experiments reported herein were designed to determine direct effects of the drug on the heart in a system capable of detecting simultaneously, alterations in mechanical, electrical and biochemical activity.

MATERIALS AND METHODS

Surgical and Perfusion Methods

Wistar male albino rats (approximately 220-250 g) given Purina Lab Chow and water *ad libitum* were used in this study. The animals were sacrificed by decapitation and perfused by the Langendorff technique with Krebs-Ringer bicarbonate buffer (K-R buffer) as previously described (Aronson and Serlick 1976, 1977a).

Analytical Methods

Hearts selected for biochemical analysis were rapidly frozen in place on the perfusion apparatus by clamping the tissue with Wollenberger tongs which had been pre-cooled in liquid nitrogen. (Wollenberger *et al*, 1960). The methods used to determine tissue metabolite concentrations and enzymatic activity have been reported in an earlier publication (Aronson and Serlick 1976).

Electrocardiographic Recording Techniques

Wick type electrodes were used to record electrical activity from the surface of the heart as previously described (Aronson and Serlick, 1977a, Aronson and Hanno, 1978). Heart rate, PR and QT intervals were determined directly from the original tracings according to previously established guidelines, and the QT interval was corrected for variability in heart rate (Aronson and Serlick, 1977b).

Statistical Methods

The standard error of the mean for each group was calculated and the data were examined by either the paired variate or independent *t* test (Ipsen and Feigl, 1970).

Drugs

The PGB_x used in these studies was synthesized and supplied by the Biochemistry Laboratory of the Naval Air Development Center, Warminster, Pennsylvania. PGB_x was made available to us in powder form as the sodium salt, and all dosages were calculated and expressed as such. The molecular weight of material which we used (Preparation #25) was taken to be 2150. All dilutions of the drug, prior to its addition to the perfusion medium, were made with physiological saline (PSS, 0.9% NaCl). PGB_x was considered stable under the storage conditions which we employed and those of our perfusion system (Polis, 1978).

RESULTS

Effect of PGB_x on Mechanical Activity in the Isolated Perfused Rat Heart

In hearts perfused with control (drug-free) medium for 60 minutes after the initial 15 minute equilibration period, diastolic tension declined slowly throughout the course of the experiment (Table 1-1). Spontaneous heart rate decreased simultaneously in this same population of hearts whereas coronary flow remained relatively constant. Isometric systolic tension developed by control hearts increased during the first 30 minutes of perfusion, but by 60 minutes returned to a value not significantly different from control.

When PGB_x was added to the medium and hearts were perfused at different concentrations (10, 100, 500 and 1000 ng/ml), alterations in coronary flow, isometric systolic tension and diastolic tension were observed. The greatest effects occurred at 500 and 1000 ng/ml respectively. At these concentrations, coronary flow and isometric systolic tension were reduced significantly whereas comparable control hearts showed no such changes. Diastolic tension decreased initially at 15 minutes,

but subsequently increased to a value considerably above its initial value in PGB_x (1000 ng/ml)-treated hearts. This increase showed statistical significance only at the highest dose, whereas at lower doses, the responses more closely resembled those which occurred in the control hearts.

Isometric systolic tension decreased in each group of hearts perfused with PGB_x-containing medium (Table 1-1), and at the highest dose (1000 ng/ml), was approximately 30% its initial control value compared to 90% in the control group.

Coronary flow (Table 1-1) remained relatively constant at low doses of PGB_x (10 and 100 ng/ml), but at higher concentrations (500 and 1000 ng/ml), it decreased to 57% and 41% of control, respectively. In control hearts, on the other hand, flow was unchanged.

Spontaneous heart rate was variable, and while decreases occurred in PGB_x-perfused hearts, a similar decrease took place in the control group (Table 1-1). It is difficult, therefore, to ascribe a negative chronotropic action to PGB_x in view of the changes observed in the control group.

Effect of PGB_x on Electrical Activity in the Isolated Perfused Rat Heart

PGB_x had no effect on the electrical activity of the perfused heart at two of the four concentrations studied in this system (Table 1-2). At 500 and 1000 ng/ml, however, an intermittant increase in the duration of the QT interval was observed, but by 60 minutes the values returned to within normal limits. The PR interval showed a transient increase at 15 and 30 minutes at 1000 ng/ml, but once again the prolongation was not significant at 45 and 60 minutes respectively.

Recordings of electrical activity showed little deviation from the pattern normally observed and no serious arrhythmias were observed consistently and/or ascribed to PGB_x. In one heart, however, AV block occurred briefly at 1000 ng/ml

(Figure 1-1) after 60 minutes of perfusion, but it reverted spontaneo sly to a normal sinus rhythm.

Effect of PGB_x on Metabolite Concentrations in the Isolated Perfused Rat Heart

At 1000 ng/ml (Table 1-3), there was a marked decrease in the concentration of tissue glycogen in the heart accompanied by an increase in the amount of D-glucose-1-PO_4 and D-glucose-6-PO_4 present in the same samples. Adenosine-5'-triphosphate (ATP), total adenine nucleotides (AMP+ADP+ATP), and creatine phosphate were also significantly reduced. The profile was similar at 500 ng/ml, but glycogen and D-glucose-1-PO_4 concentrations were not altered significantly compared to hearts perfused only with drug-free medium.

Phosphorylase *a* activity was not elevated from control (14.1 $\pm$ 1.2%) by PGB_x at the concentrations studied after one hour of perfusion with the drug, although the diminished glycogen concentration which occurred at 1000 ng/ml (Table 1-3) suggests that enhanced activity of this system probably occurred earlier in time during the course of the experiment.

The amount of lactate found in coronary effluents was relatively constant in control hearts and at the lower doses of PGB_x, but at 500 and 1000 ng/ml was considerably elevated above control values by 60 minutes.

DISCUSSION

The ability of PGB_x to restore oxidative phosphorylation and enhance ATP synthesis in aged and/or damaged mitochondria is unique (Polis *et al*, 1973; Devlin, 1978), and its lack of effect on the metabolism of normal mitochondria suggests that perhaps it does not gain access to target sites under "normal" conditions.

Recent work, however, has shown PGB_x to have a biphasic nature, that is to stimulate aged mitochondria at lower doses and to inhibit their metabolism at higher concentrations (Devlin, 1978).

Our findings in the isolated perfused rat heart are consistant, in this regard, with those reported in mitochondria, since we found the drug had no effect in normal hearts at low concentrations (10 and 100 ng/ml), but appeared to depress the heart at higher concentrations (500 and 1000 ng/ml). At the latter concentrations, its biochemical effects closely resembled those produced in isolated perfused rat hearts by disophenol and bunamidine, veterinary anthelmintics (Aronson and Serlick, 1977b; Aronson and Hanno, 1978), and di-2-ethyl hexyl phthalate (DEHP), a plasticizer (Aronson *et al*, 1978). Each of these agents markedly enhanced glycogen utilization and lowered the concentration of ATP and total adenine nucleotides (ATP+ADP+AMP) in the heart in addition to modifying similarly the concentrations of several other metabolites.

The effects on mechanical activity produced by PGB_x at 1000 ng/ml are similar to those caused by disophenol, bunamidine and DEHP in that these drugs depressed spontaneous heart rate, coronary flow and isometric systolic tension while they also increased diastolic tension at high concentrations. In aged mitochondria from rat liver, Polis (1977) demonstrated that disophenol inhibited this system in a manner analogous to 2,4-dinitrophenol, and PGB_x produced a dose dependent reversal of this inhibition when added to the system. The specific site and mechanism of this antagonism between disophenol and PGB_x in mitochondria remains unclear. However, since both drugs depressed the heart and produced similar biochemical effects in our system, we plan, in future experiments, to determine whether and how they interact in the isolated perfused rat heart. Our objective will be to determine whether PGB_x can effectively antagonize and/or protect against disophenol-induced alterations in mechanical, electrical and biochemical functions.

REFERENCES

Angelakos, E. T., Riley, R. L. and Polis, B. D., (1977) Recovery of monkeys from cardiogenic shock after myocardial infarction with ventricular fibrillation, Effects of PGB_x Report No. NADC-77308-60, Office of Naval Research, Arlington.

Aronson, C. E. and Serlick, E. R., (1976) Effects of prolonged perfusion time on the isolated perfused rat heart, *Toxic. Appl. Pharmac.* 38,479-488.

Aronson, C. E. and Hanno, E. R. S., (1978) Effects of bunamidine on the isolated perfused rat heart, *Gen. Pharmac.* 9,101-112.

Aronson, C. E. and Serlick, E. R., (1977a) Effects of chlorpromazine on the isolated perfused rat heart, *Toxic. Appl. Pharmac.* 39,157-176.

Aronson, C. E. and Serlick, E. R., (1977b) Effects of disophenol on the isolated perfused rat heart, *Biochem. Pharmac.* 2297-2305.

Aronson, C. E., Serlick, E. R. and Preti, G., (1978) Effects of Di-2-ethylhexyl phthalate on the isolated perfused rat heart, *Toxic. Appl. Pharmac.* 44,155-169.

Devlin, T., (1978) Personal communication.

Ipsen, J. and Feigl, P., (1970) *Bancroft's Introduction to Biostatistics*, 2nd edn., Harper and Row, New York.

Polis, B. D., Grandizio, A. M. and Polis, E., (1973) Some *in vitro* and *in vivo* effects of a new prostaglandin derivative in *Neurohumoral and Metabolic Aspects of Injury*; (Kovack, A. G. B., Stoner, H. B. and Spitzer, J. J., Eds.) Plenum Publishing Corp., New York.

Polis, E., (1978) Personal communication.

Polis, B. D., (1977) Personal communication, unpublished data.

Riley, R. L., Polis, D. and Angelakos, E. T., (1974) Protective effect of a prostaglandin derivative on mortality following coronary ligation and ventricular fibrillation, *Physiologist* 17,320.

Wollenberger, A., Ristau, O. and Schoffa, G., (1960) Eine einfache Technik der extrem schnellen Abkuhlung grösserer gewebestüke, *Pflugers Arch. ges Physiol.* 270,399-412.

TABLE 1-1

EFFECT OF PGB_x ON MECHANICAL ACTIVITY OF THE ISOLATED PERFUSED RAT HEART[a]

Drug	Concentration[b]	N[c]	Time	Spontaneous Heart Rate (bpm)	Coronary Flow (ml/min)	Isometric Systolic Tension (g)	Diastolic Tension (g)
None	-	5	0	246 ± 13.2	9.3 ± 1.0	15.3 ± 0.9	4.6 ± 0.2
			15	220 ± 13.6[d]	9.2 ± 1.6	17.5 ± 1.1[d]	4.2 ± 0.1[d]
			30	218 ± 17.5[d]	9.4 ± 1.3	17.3 ± 1.1[d]	3.8 ± 0.2[d]
			45	218 ± 17.4[d]	10.2 ± 1.2	16.1 ± 0.7	3.6 ± 0.3[d]
			60	203 ± 21.9[d]	9.9 ± 1.4	13.7 ± 0.7	3.2 ± 0.3[d]
PGB_x	10 ng/ml	5	0	290 ± 16.1	7.6 ± 0.4	15.9 ± 0.5	5.0 ± 0.2
			15	290 ± 19.3	7.4 ± 0.4	15.8 ± 0.7	4.5 ± 0.2[d]
			30	284 ± 15.6	7.5 ± 0.5	15.2 ± 0.7	4.1 ± 0.1[d]
			45	274 ± 18.1	7.7 ± 0.4	14.7 ± 0.8	4.0 ± 0.1[d]
			60	272 ± 17.4	7.5 ± 0.8	13.4 ± 0.7[d]	3.9 ± 0.1[d]
PGB_x	100 ng/ml	5	0	257 ± 13.3	7.4 ± 0.5	14.8 ± 1.3	4.8 ± 0.1
			15	243 ± 9.1	7.0 ± 0.5	13.4 ± 1.7	4.3 ± 0.4
			30	231 ± 12.3[d]	7.9 ± 0.7	13.5 ± 0.8	4.1 ± 0.4
			45	236 ± 12.1[d]	7.0 ± 0.5	12.4 ± 0.8[d]	4.1 ± 0.4
			60	226 ± 10.9[d]	6.7 ± 0.5	10.4 ± 1.0[d]	4.1 ± 0.4
PGB_x	500 ng/ml	7	0	255 ± 9.2	7.5 ± 0.9	13.9 ± 0.7	4.7 ± 0.2
			15	234 ± 12.8[d]	7.5 ± 1.0	14.4 ± 0.3	4.2 ± 0.2[d]
			30	231 ± 14.6	6.3 ± 0.7	11.6 ± 1.2	4.3 ± 0.3
			45	213 ± 17.4[d]	5.3 ± 0.6[d]	8.7 ± 1.5[d]	4.8 ± 0.4
			60	198 ± 18.8[d]	4.3 ± 0.6[d]	6.8 ± 1.3[d]	5.1 ± 0.4
PGB_x	1000 ng/ml	5	0	244 ± 9.8	7.3 ± 0.8	16.9 ± 1.3	4.9 ± 0.1
			15	244 ± 13.7	6.8 ± 0.2	16.0 ± 1.0	4.3 ± 0.1[d]
			30	221 ± 14.6[d]	5.0 ± 0.3	11.5 ± 2.0	4.8 ± 0.3
			45	205 ± 22.5	3.9 ± 0.5	7.2 ± 1.0[d]	5.6 ± 0.3
			60	193 ± 19.1d	3.0 ± 0.3[d]	5.0 ± 0.4[d]	6.4 ± 0.1[d]

[a] Hearts obtained from normal male rats (220-250 g).
[b] PGB_x calculated and expressed as the sodium salt. Hearts were perfused with control or PGB_x-containing medium for 60 minutes after the initial 15 minute equilibration period.
[c] Number of hearts in each group.
[d] Significant ($P<0.05$) compared to 0 perfusion time within each group by paired variate t test.

TABLE 1-2

EFFECT OF PGB_x ON ELECTRICAL ACTIVITY OF THE ISOLATED PERFUSED RAT HEART[a]

Drug	Concentration[b]	N[c]	Time	PR (ms)	QT (ms)	QTc[d]
None	-	5	0	45 ± 1.4	81 ± 3.3	5.16 ± 0.23
			15	44 ± 1.8	83 ± 2.0	5.01 ± 0.16
			30	46 ± 1.9	84 ± 2.4	5.03 ± 0.11
			45	45 ± 2.1	84 ± 1.8	5.01 ± 0.14
			60	44 ± 1.6	84 ± 2.4	4.83 ± 0.13
PGB_x	10 ng/ml	5	0	44 ± 2.0	76 ± 1.9	4.84 ± 0.30
			15	44 ± 1.8	77 ± 2.0	4.93 ± 0.33
			30	45 ± 1.5	74 ± 2.5	4.85 ± 0.33
			45	44 ± 1.6	75 ± 2.2	4.73 ± 0.30
			60	44 ± 1.8	71 ± 3.3	4.76 ± 0.37
PGB_x	100 ng/ml	5	0	42 ± 1.0	70 ± 3.2	4.59 ± 0.16
			15	42 ± 1.0	70 ± 3.2	4.47 ± 0.16
			30	42 ± 1.0	72 ± 4.9	4.46 ± 0.21
			45	41 ± 0.8	73 ± 5.5	4.55 ± 0.26
			60	43 + 1.9	73 ± 5.5	4.45 ± 0.30
PGB_x	500 ng/ml	7	0	45 ± 1.5	79 ± 1.4	5.13 ± 1.13
			15	45 ± 1.1	83 ± 2.9	5.17 ± 0.22
			30	46 ± 1.1	84 ± 3.0	5.22 ± 0.25
			45	47 ± 0.9	86 ± 3.7	5.08 ± 0.31
			60	47 ± 1.4	86 ± 4.3	4.90 ± 0.38
PGB_x	1000 ng/ml	5	0	46 ± 1.9	78 ± 4.9	4.97 ± 0.24
			15	50 ± 1.9[e]	88 ± 8.6	5.55 ± 0.40
			30	52 ± 2.6[e]	88 ± 7.3[e]	5.29 ± 0.32
			45	62 ± 9.9	84 ± 5.1	4.82 ± 0.15
			60	66 ± 11.6	84 ± 5.1	4.70 ± 0.21

[a] Hearts obtained from normal male rats (220-250 g).

[b] PGB_x calculated and expressed as the sodium salt. Hearts were perfused with control or PGB_x-containing medium for 60 minutes after the initial 15 minute equilibration period.

[c] Number of hearts in each group.

[d] $QTc = \frac{QT\ (ms)}{\sqrt{R\text{-}R\ (ms)}}$

[e] Significant ($P \leq 0.05$) compared to 0 perfusion time within each group by paired variate *t* te

TABLE 1-3

EFFECT OF PGB_x ON METABOLITE CONCENTRATIONS IN THE ISOLATED PERFUSED RAT HEART[a]

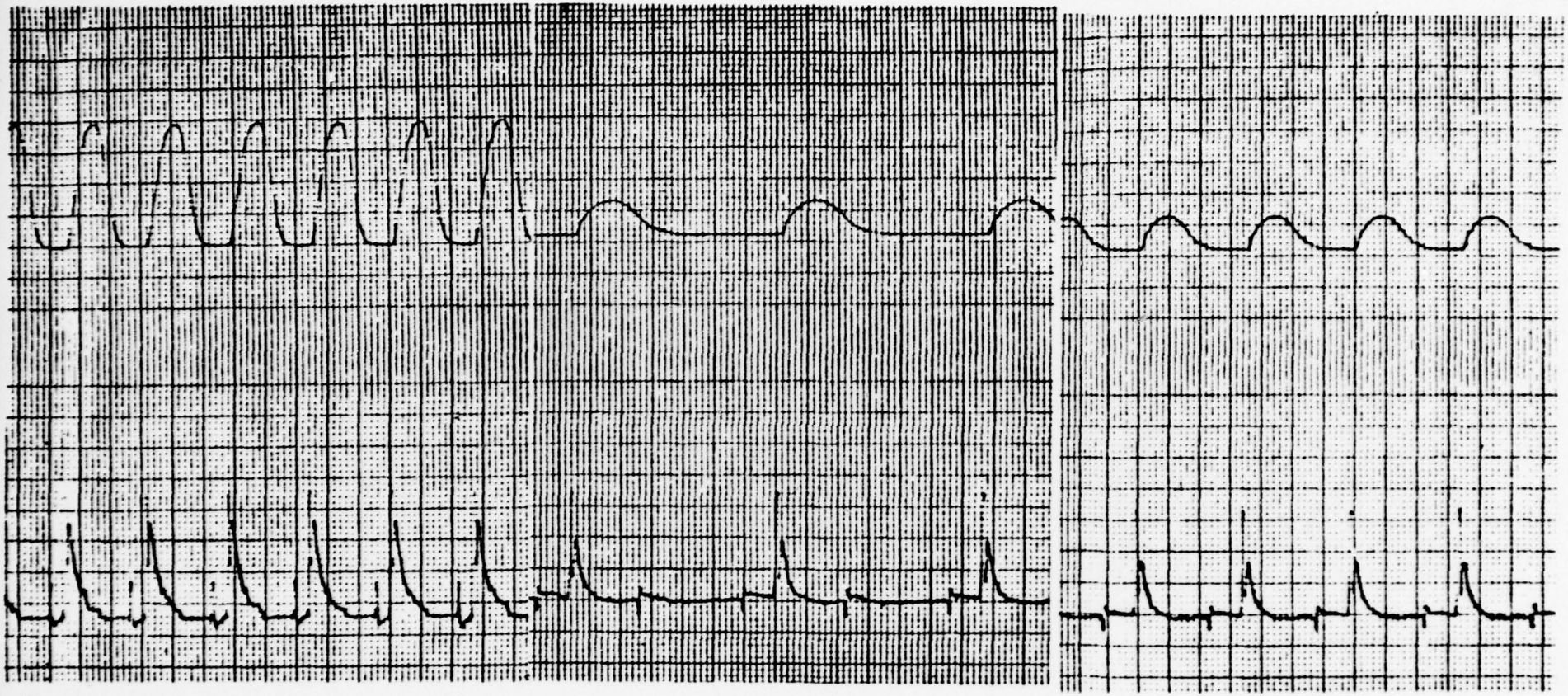
Metabolite	Concentration of PGB_x in Perfusion Medium[b]									
	N[c]	0 ng/ml μM/g ± SEM[d]	N[c]	10 ng/ml μM/g ± SEM[d]	N[c]	100 ng/ml μM/g ± SEM[d]	N[c]	500 ng/ml μM/g ± SEM[d]	N[c]	1000 ng/ml μM/g ± SEM[d]
Glycogen	5	13.51 ± 1.43	5	11.02 ± 1.08	5	12.65 ± 1.45	7	11.13 ± 1.74	5	6.92 ± 1.04[e]
D-Glucose-1-PO_4	5	0.0000 ± 0.0000	5	0.0000 ± 0.0000	5	0.0000 ± 0.0000	7	0.0099 ± 0.0057	5	0.0260 ± 0.095[e]
D-Glucose-6-PO_4	5	0.0200 ± 0.0034	5	0.0191 ± 0.0068	5	0.0346 ± 0.0087	7	0.0690 ± 0.0173[e]	5	0.0976 ± 0.0116[e]
D-Fructose-6-PO_4	5	0.0083 ± 0.0051	5	0.0120 ± 0.0042	5	0.0120 ± 0.0068	7	0.0289 ± 0.0169	5	0.0156 ± 0.0064
D-Fructose-1,6-di PO_4	5	0.0360 ± 0.0034	5	0.0332 ± 0.0069	5	0.0243 ± 0.0067	7	0.0391 ± 0.0047	5	0.0487 ± 0.0158
Dihydroxyacetone PO_4	5	0.1210 ± 0.0103	5	0.1044 ± 0.0115	5	0.0577 ± 0.0158	7	0.1197 ± 0.0114	5	0.1026 ± 0.0211
D-Glyceraldehyde-3-PO_4	5	0.0648 ± 0.0144	5	0.0601 ± 0.0101	5	0.0546 ± 0.0119	7	0.1038 ± 0.0219	5	0.1185 ± 0.0233
L-(-)-Glycerol-1-PO_4	5	0.2366 ± 0.0409	5	0.2939 ± 0.0504	5	0.3661 ± 0.0580	7	0.2878 ± 0.0471	5	0.3863 ± 0.0998
Pyruvate	5	0.1066 ± 0.0542	5	0.1574 ± 0.0527	5	0.1142 ± 0.0454	7	0.1509 ± 0.0295	5	0.1680 ± 0.0481
L-(+)-Lactate	5	0.7330 ± 0.1337	5	0.4657 ± 0.0528	5	0.8825 ± 0.2592	7	1.2970 ± 0.3905	5	1.3578 ± 0.2934
Adenosine-5'-tri PO_4	5	2.4805 ± 0.2484	5	2.2926 ± 0.0778	5	2.1160 ± 0.1199	7	1.3287 ± 0.1889[e]	5	1.1883 ± 0.0814[e]
Adenosine-5'-di PO_4	5	0.2890 ± 0.0546	5	0.3676 ± 0.0617	5	0.3015 ± 0.0356	7	0.2411 ± 0.0380	5	0.2531 ± 0.0473
Adenosine-5'-mono PO_4	5	0.0715 ± 0.0110	5	0.0955 ± 0.0173	5	0.0953 ± 0.0108	7	0.0714 ± 0.0141	5	0.0470 ± 0.0141
Total Adenine Nucleotides	5	2.8411 ± 0.2760	5	2.7557 ± 0.0832	5	2.5128 ± 0.1143	7	1.6412 ± 0.1975[e]	5	1.4885 ± 0.0922[e]
Creatine-PO_4	5	2.4874 ± 0.2334	5	2.2272 ± 0.1820	5	2.0056 ± 0.2437	7	1.6413 ± 0.1550[e]	5	1.5881 ± 0.1446[e]

[a] Hearts obtained from normal male rats (220-250 g).
[b] PGB_x calculated and expressed as the sodium salt. Hearts were perfused with control or PGB_x-containing medium for 60 minutes after the initial 15 minute equilibration period.
[c] Number of hearts in each group.
[d] Expressed per gram of tissue (wet weight).
[e] Significant ($P \leq 0.05$) compared to control (0 drug concentration) group by an independant t test.

FIGURE LEGEND

Figure 1-1

These recordings were made from a heart perfused with Krebs-Ringer Bicarbonat buffer containing PGB_x (1000 ng/ml). Isometric systolic tension (1 g/mm) is shown in the upper part of each tracing, while electrical activity (2 mV/cm) is shown below. Paper speed was 50 mm/sec. The first or 0 time recording (A) was made at the end of the 15-minute equilibration period with drug-free buffer, after which the heart was perfused with medium containing PGB_x. After 60 minutes of perfusion with PGB_x, the heart developed a transient 2:1 AV block (B) which reverted spontaneously to a normal sinus rhythm (C).



(A) (B) (C)

FIGURE 1-1

PART II

INTRODUCTION

In Part I of this report, data were presented which showed that PGB_x produced a dose related depression of isometric systolic tension and coronary flow in the isolated perfused rat heart. PGB_x also altered glycogen metabolism and ATP production in these same hearts.

Disophenol, an anthelmintic drug, produced similar effects in this preparation (Aronson and Serlick, 1977), but Polis (1977) found that PGB_x antagonized the inhibitory action of disophenol in aged mitochondria (prepared from liver). Eichel (1979), in related experiments, confirmed the earlier work of Polis (1977) in aged mitochondria which showed disophenol to be a true inhibitor of oxidative phosphorylation. He found, however, that PGB_x was limited in its ability to counteract and reverse the disophenol-induced depression of mitochondrial oxidative phosphorylation in preparations of aged mitochondria. Disophenol also inhibited oxidative phosphorylation in fresh preparations of mitochondria, but had a greater effect in the aged preparation (Eichel, 1979).

The studies presented in this section of the report (Part II) were conducted to determine whether pre-perfusion with PGB_x (100 ng/ml) would affect the deleterious action of disophenol on well oxygenated normal hearts.

MATERIALS AND METHODS

The surgical, perfusion, rapid freezing and analytical techniques described and referenced in Part I of this report were used also in the experiments reported in Part II.

Disophenol (40 µg/dose) was injected as a bolus into the flow of perfusion fluid entering the heart at exactly 5 minutes and 10 minutes after the initial 15 minute equilibration period with drug-free K-R buffer. In those hearts perfused with PGB_x-containing medium (100 ng/ml), PGB_x began entering the heart immediately upon the conclusion of the 15 minute equilibration period and continued through the course of the experiment. These hearts, therefore, were pre-perfused with PGB_x for exactly 5 minutes before receiving their first dose of disophenol.

The 100 ng/ml concentration of PGB_x was selected because it produced no evidence of electrical or biochemical disturbance in the heart. The 40 µg dose of disophenol, administered as a bolus, was determined experimentally as one which would cause an almost immediate depression upon injection, but generally not cardiac arrest.

The disophenol (DNP®) used in these studies was a gift from the American Cyanamid Company, Princeton, New Jersey.

RESULTS

Effect of PGB_x and Disophenol on Mechanical Activity of the Isolated Perfused Rat Heart

In hearts perfused with disophenol and PGB_x, spontaneous heart rate responded no differently than hearts in the control group which showed a similar decrease in rate 30 minutes after the initial 15 minute equilibration period (Table 2-1).

Coronary flow, however, increased significantly after hearts received disophenol alone, but PGB_x appeared to alter this response since flow remained unchanged when disophenol was administered in hearts perfused simultaneously with PGB_x (100 ng/ml)-containing buffer (Table 2-1).

DISCUSSION

In preliminary experiments by Polis (1977) and those conducted more recently by Eichel (1979), it appeared that PGB_x had a measure of effectiveness in restoring oxidative phosphorylation towards normal in disophenol-inhibited preparations of aged mitochondria prepared from liver.

In the well oxygenated perfused rat heart, disophenol depressed mechanical, electrical and biochemical activity of this preparation when the hearts were perfused with disophenol-containing medium at various concentrations for periods up to 60 minutes (Aronson and Serlick, 1977). In the experiments reported herein, our primary objective was to determine whether the effect of a disophenol-induced inhibition of normal activity in the isolated perfused rat heart would be altered by the presence of PGB_x. The data in Part II of this report showed little effect of PGB_x under the conditions of our experiments. PGB_x did, however, appear to interfere with the increase in coronary flow observed when only disophenol was administered. From these experiments alone, it was not possible to identify the mechanism involved, and it would be necessary to conduct further experiments, perhaps using isolated vessels to determine the effects of such interaction directly on the blood vessels.

By inspection of the data, disophenol, in the presence of PGB_x, appeared to have a greater inhibitory action on adenine nucleotides, and to promote greater glycogen utilization; the respective values showed no difference between these groups. One would, nevertheless, perhaps spectulate, simply by inspection, that PGB_x was having an additive rather than an antagonistic effect as far as the disophenol-induced inhibition of ATP production and the stimulation of glycogen utilization were concerned. One might postulate also that perhaps both drugs were acting upon the same and/or similar sites.

Isometric systolic tension decreased significantly in PGB_x-disophenol treated hearts and did not return to pre-disophenol values by the end of the prescribed perfusion period. In those hearts receiving disophenol in conjunction with drug-free perfusion medium, isometric systolic tension tended to decrease as well, but the decrease observed was not significant (Table 2-1). Diastolic tension, on the other hand, increased significantly in the group treated with disophenol and PGB_x whereas the response in hearts receiving disophenol alone (Table 2-1) was not significant.

Effects of PGB_x and Disophenol on Biochemical Activity of the Isolated Perfused Rat Heart

Disophenol decreased tissue concentrations of glycogen in both drug-free and PGB_x (100 ng/ml)-perfused hearts when compared to the control group which received neither agent (Table 2-2). There was, however, no significant difference ($P>0.4$) between the glycogen content of those hearts which received disophenol alone and those which received it in the presence of PGB_x. The same was also true for ATP ($P>0.4$), AMP ($P>0.5$) and the total adenine nucleotide concentrations (>0.2). ADP concentrations were, however, lower ($P<0.05$) in the PGB_x-perfused hearts which received disophenol.

The disophenol-induced increase in lactate in the coronary effluent was also not statistically significant in either group (Table 2-3), nor was the absolute difference in lactate produced between these same groups ($P>0.1$). Phosphorylase *a* activity was the same in each group at the time of sampling (Table 2-4).

Whereas most of the mitochondria used to study PGB_x have been prepared from liver, it would be of interest, certainly, to determine whether or not mitochondria prepared from rat hearts responded in the same characteristic manner. It might be that in heart mitochondria the nature and/or site of the action of PGB_x is different than that which occurred characteristically in liver preparations, and this possibility should be explored.

REFERENCES

Aronson, C. E. and Serlick, E. R. (1977) Effects of disophenol on the isolated perfused rat heart, *Biochem. Pharmac.* 2297-2305.

Eichel, H. E. (1979) Personal communication and unpublished data.

Polis, B.D. (1977) Personal communication, unpublished data.

TABLE 2-1

EFFECTS OF PGB_x AND DISOPHENOL ON MECHANICAL ACTIVITY OF THE ISOLATED PERFUSED RAT HEART[a]

Drug	Time Sequence	N[g]	Spontaneous Heart Rate	P[h]	Coronary Flow	P[h]	Isometric Systolic Tension	P[h]	Diastolic Tension	P[h]
None	A[d]	6	275 ± 25.4	-	6.8 ± 0.4	-	13.5 ± 1.2	-	4.7 ± 0.2	-
	B[e]	6	267 ± 21.7	>0.4	6.4 ± 0.4	<0.005	14.1 ± 1.2	>0.5	4.5 ± 0.2	>0.05
	C[f]	6	246 ± 19.6	<0.05	7.1 ± 0.4	>0.4	14.3 ± 1.3	>0.1	4.1 ± 0.2	<0.01
PGB_x[b]	A[d]	5	275 ± 12.0	-	8.0 ± 0.4	-	15.7 ± 1.7	-	4.5 ± 0.1	-
	B[e]	5	271 ± 9.1	>0.5	7.5 ± 0.2	>0.5	15.8 ± 1.5	>0.5	4.4 ± 0.3	>0.5
	C[f]	5	251 ± 12.2	<0.05	8.1 ± 0.3	>0.2	16.0 ± 1.4	>0.5	4.0 ± 0.3	>0.1
Disophenol[c]	A[d]	5	272 ± 15.0	-	7.4 ± 0.5	-	14.3 ± 0.9	-	4.5 ± 0.1	-
	B[e]	5	196 ± 50.5	>0.4	10.0 ± 0.6	<0.005	8.7 ± 2.4	>0.05	8.6 ± 1.9	>0.05
	C[f]	5	198 ± 49.9	>0.1	8.2 ± 0.6	>0.1	11.4 ± 3.1	>0.2	6.8 ± 2.1	>0.2
PGB_x + Disophenol[c]	A[d]	5	266 ± 5.5	-	8.4 ± 0.4	-	14.6 ± 0.7	-	4.7 ± 0.2	-
	B[e]	5	228 ± 27.4	>0.1	8.6 ± 1.6	>0.2	5.7 ± 2.4	<0.01	10.6 ± 1.3	<0.025
	C[f]	5	241 ± 10.7	<0.05	7.7 ± 0.3	>0.2	10.4 ± 2.0	<0.05	7.1 ± 1.3	>0.1

[a] Hearts obtained from normal male rats (220-250 g).
[b] PGB_x calculated and expressed as the sodium salt. Concentration in K-R buffer = 100 ng/ml. Flow started at end of 15 minute equilibration period.
[c] Disophenol (40 µg) injected as a bolus into the flow of perfusion fluid entering the heart at 5 and 10 minutes after initial 15 minute equilibration period.
[d] A = 5 minutes after initial 15 minute equilibration period.
[e] B = 15 minutes after initial 15 minute equilibration period.
[f] C = 30 minutes after initial 15 minute equilibration period.
[g] Number of hearts in each group.
[h] Compared to A in each group by paired variate *t* test. P values ≤0.05 were considered significant .

TABLE 2-2

EFFECTS OF PGB_x AND DISOPHENOL ON METABOLITE CONCENTRATIONS IN THE ISOLATED PERFUSED RAT HEART[a]

Metabolite	Control[b]		PGB_x[b]			Disophenol[b]			PGB_x + Disophenol[b]		
	N[c]	µM/g[d] ± S.E.M.	N[c]	µM/g[d] ± S.E.M.	P[e]	N[c]	µM/g[d] ± S.E.M.	P[e]	N[c]	µM/g[d] ± S.E.M.	P[e]
Glycogen	6	18.07 ± 1.02	5	15.99 ± 0.57	>0.05	5	12.40 ± 1.95	<0.025	5	9.70 ± 2.87	<0.025
D-Glucose-1-PO_4	6	0.0062 ± 0.0032	5	0.0019 ± 0.0014	>0.1	5	0.0074 ± 0.0035	>0.5	5	0.0037 ± 0.0024	>0.4
L-(-)-Glycerol-1-PO_4	6	0.1140 ± 0.0244	5	0.1349 ± 0.0183	>0.5	5	0.1442 ± 0.0489	>0.5	5	0.1183 ± 0.0512	>0.5
L-(+)-Lactate	6	0.4652 ± 0.0578	5	0.4864 ± 0.0572	>0.5	5	0.3868 ± 0.1113	>0.5	5	0.3961 ± 0.1567	>0.5
Adenosine-5'-tri PO_4	6	2.9761 ± 0.1518	5	2.4550 ± 0.1640	<0.05	5	2.1872 ± 0.2528	<0.025	5	1.9491 ± 0.1956	<0.005
Adenosine-5'-di PO_4	6	0.5602 ± 0.0537	5	0.5567 ± 0.0329	>0.5	5	0.4316 ± 0.0371	>0.05	5	0.3036 ± 0.0342	<0.005
Adenosine-5'-mono PO_4	6	0.0510 ± 0.0050	5	0.0903 ± 0.0183	<0.05	5	0.1852 ± 0.0517	<0.025	5	0.1528 ± 0.0535	>0.05
Total Adenine Nucleotides	6	3.5872 ± 0.1803	5	3.1021 ± 0.1924	>0.05	5	2.8040 ± 0.2413	<0.05	5	2.4055 ± 0.2028	<0.005
Creatine PO_4	6	2.2098 ± 0.1583	5	1.8774 ± 0.1410	>0.1	5	2.3163 ± 0.1815	>0.5	5	2.8400 ± 0.3337	>0.1

[a] Hearts obtained from normal male rats (220-250 g)
[b] Hearts frozen 30 minutes after initial 15 minute equilibration period at the end of time sequence C as defined in footnotes to Table 1. Drugs, their concentrations and mode of administration are as stated in the footnotes to Table 1.
[c] Number of hearts in each group.
[d] Expressed per gram of tissue (wet weight)
[e] Compared to control (drug-free) group by an independent *t* test. P values ≤0.05 were considered significant.

TABLE 2-3

EFFECTS OF PGB_x AND DISOPHENOL ON LACTATE CONCENTRATIONS IN CORONARY EFFLUENTS[a]

Drug[b]	Time Sequence[c]	N[d]	Lactate[e] (μM/g/min)	P[f]
None	A	6	0.282 ± 0.028	-
	B		0.275 ± 0.027	>0.5
	C		0.240 ± 0.030	>0.1
PGB_x	A	5	0.287 ± 0.071	-
	B		0.235 ± 0.044	>0.1
	C		0.218 ± 0.057	<0.05
Disophenol	A	5	0.272 ± 0.068	-
	B		0.409 ± 0.079	>0.2
	C		0.306 ± 0.117	>0.5
PGB_x + Disophenol	A	5	0.265 ± 0.047	-
	B		1.006 ± 0.366	>0.1
	C		0.225 ± 0.036	>0.2

[a] Hearts obtained from normal male rats (220-250 g).
[b] Drugs, their concentrations and mode of administration are identical to those described in the footnotes to Table 1.
[c] Time sequences are identical to those described in the footnotes to Table 1.
[d] Number of hearts in each group.
[e] Calculated on the basis of tissue wet weight.
[f] Compared to A in each group by paired variate *t* test. P values ≤0.05 were considered significant.

TABLE 2-4

EFFECTS OF PGB_x AND DISOPHENOL ON CARDIAC PHOSPHORYLASE *a* ACTIVITY[a]

Drug[b]	N[c]	% Phosphorylase *a*[d]	P[e]
None	6	15.1 ± 1.63	-
PGB_x	5	15.3 ± 1.23	>0.5
Disophenol	5	14.1 ± 1.44	>0.5
PGB_x + Disophenol	5	13.7 ± 1.27	>0.5

[a] Hearts obtained from normal male rats (220-250 g).

[b] Hearts frozen 30 minutes after initial 15 minute equilibration period at the end of time sequence C as defined in footnotes to Table 1. Drugs, their concentrations and mode of administration are as stated in the footnotes to Table 1.

[c] [d] Number of hearts in each group.

$$\% \text{ Phosphorylase } a = \frac{\text{Cori Units of Phosphorylase } a}{\text{Total Cori Units } (a+b)} \times 100$$

[e] Compared to control (drug-free) group by an independent *t* test. P values ≤0.05 were considered.